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Autodisplay: efficient bacterial surface display of recombinant proteins

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Abstract To display a protein or peptide with a distinct function at the surface of a living bacterial cell is a challenging exercise with constantly increasing impact in many areas of biochemistry and biotechnology. Among other systems in Gram-negative bacteria, the Autodisplay system provides striking advantages when used to express a recombinant protein at the surface of *Escherichia coli* or related bacteria. The Autodisplay system has been developed on the basis of and by exploiting the natural secretion mechanism of the AIDA-I autotransporter protein. It offers the expression of more than 10^5 recombinant molecules per single cell, permits the multimerization of subunits expressed from monomeric genes at the cell surface, and allows, after transport of an apoprotein to the cell surface, the incorporation of an inorganic prosthetic group without disturbing cell integrity or cell viability. Moreover, whole cells displaying recombinant proteins by Autodisplay can be subjected to high-throughput screening (HTS) methods such as ELISA or FACS, thus enabling the screening of surface display libraries and providing access to directed evolution of the recombinant protein displayed at the cell surface. In this review, the application of the Autodisplay system for the surface display of enzymes, enzyme inhibitors, epitopes, antigens, protein and peptide libraries is summarised and the perspectives of the system are discussed.

Introduction

In many biotechnological applications, the cellular surface display of a protein or peptide with a specific function has convincing benefits (Wernerus and Stahl 2004). First, the molecule displayed at the cell surface is freely accessible

for any kind of binding or activity studies without the need for a substrate or binding partner to cross a membrane barrier. Second, when connected to a matrix, as in this case the cell envelope, proteins have proven to be more stable than free molecules. Third, the need for preparation or purification of molecules for many applications is unnecessary as whole cells displaying the molecule of interest can be applied to reactions or analytical assays and can be removed afterwards by a simple centrifugation step. Finally, cellular surface display has another significant advantage when used for creating and screening peptide or protein libraries in order to perform directed molecular evolution. By selecting the correct structure expressed at the surface, the cell with the corresponding gene, which serves as an intrinsic label, is co-selected and can be used for rapid sequence determination, first steps in structure prediction, and in other further studies or applications (Jose et al. 2005). For similar purposes, the phage display system has been developed in the mid-1980s, allowing the expression of peptides and small proteins in the envelope of filamentous bacteriophages by fusion with the pIII coat protein (Smith 1985). But cellular surface display systems may provide additional advantages as bacterial cells, in contrast to bacteriophages, are self-replicative and are sufficiently sized to be analysed by optical methods, including fluorescence microscopy or high-throughput methods as FACS (fluorescence-activated cell sorting) (Francisco et al. 1993; Wentzel et al. 1999; Bessette et al. 2004). From this point of view, it appears evident that starting with the first reports about bacterial display of heterologous proteins in 1986 by Freudl et al. (1986) and Charbit et al. (1986), a broad number of different display systems has been established for yeast, Gram-positive and Gram-negative bacteria. These systems were used in a wide range of biotechnological and industrial applications and led to substantial progress in whole cell biocatalysis, live-vaccine development, biosorbents and biosensor development, epitope mapping, antigen delivery, inhibitor design and protein/peptide library screening (for an overview see Georgiou et al. 1997; Benhar 2001; Lee et al. 2003; Wernerus and Stahl 2004). Among others, e.g. OmpA

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(Charbit et al. 1986; Freudl et al. 1986; Bessette et al. 2004) or intimin from enterohemorrhagic *Escherichia coli* (Wentzel et al. 1999), the most frequently used carrier for the surface display of recombinant proteins in *E. coli* is a chimeric protein of Lpp and OmpA. It consists of the LPP region (amino acids 1–9 of LPP) combined with the transmembrane regions B3–B7 of OmpA to which the recombinant passenger is C-terminally attached. It has been successfully applied to the expression of enzymes, single-chain antibody fragments (scFv), and binding domains at the surface of *E. coli*, but appears to be sensitive to extensive secondary and tertiary structures of the passenger protein (Georgiou et al. 1997). In Gram-positive bacteria, surface proteins are usually covalently linked to the peptidoglycan cell wall by a C-terminal anchoring tail. Therefore most of the surface display systems for recombinant proteins that have been applied in Gram-positive bacteria consist of such a C-terminal anchoring sequence to which the passenger is N-terminally attached and a typical signal sequence at the very N terminus for transport across the cytoplasmic membrane (Wernerus and Stahl 2004). The cell wall anchoring regions from different naturally occurring surface proteins, including SpA (*Staphylococcus aureus* protein A), M6 protein (*Streptococcus pyogenes*) and FnBPB (fibronectin-binding protein B from *S. aureus*), have been used for the successful display of enzymes, epitopes and binding domains at the surface of *Staphylococcus xylosus*, *Staphylococcus carnosus* and *Lactobacillus lactis* (Wernerus and Stahl 2004). These strategies take advantage of the robust cell envelope in Gram-positive bacteria, allowing the surface display of large polypeptides. Depending on the application, the covalent linkage to the peptidoglycan as well as a significantly reduced number of recombinant passenger proteins displayed per cell in comparison to systems in Gram-negative bacteria (Francisco et al. 1993; Strauss and Gotz 1996) can be a limitation.

A single display system that can fulfil all the requirements mentioned above needs to possess some essential basic features. Such a system should exhibit as less restrictions as possible concerning the size and structure of the protein displayed, the so-called passenger. Standard molecular biology protocols and standard strains should be sufficient, as well as tools that need to be on hand, thus allowing the analysis and evaluation of the passenger's display in terms of efficiency. Finally, the system should be simple and easy to handle, in order to avoid any unexpected obstacles. In order to fulfil these requirements as good as possible, the Autodisplay system has been developed (Maurer et al. 1997), which uses the secretion mechanism of the autotransporter family of proteins (Jose et al. 1995) and applies the transporting domains of the *E. coli* autotransporter protein AIDA-I (Benz and Schmidt 1989) as a carrier for recombinant passengers. In the present article, the basic structural features and the advantages of this surface display system in *E. coli* are summarised along with examples for its successful application in enzyme display, whole cell biocatalysis, inhibitor display and design, library screening and live-vaccine development.

The Autodisplay system

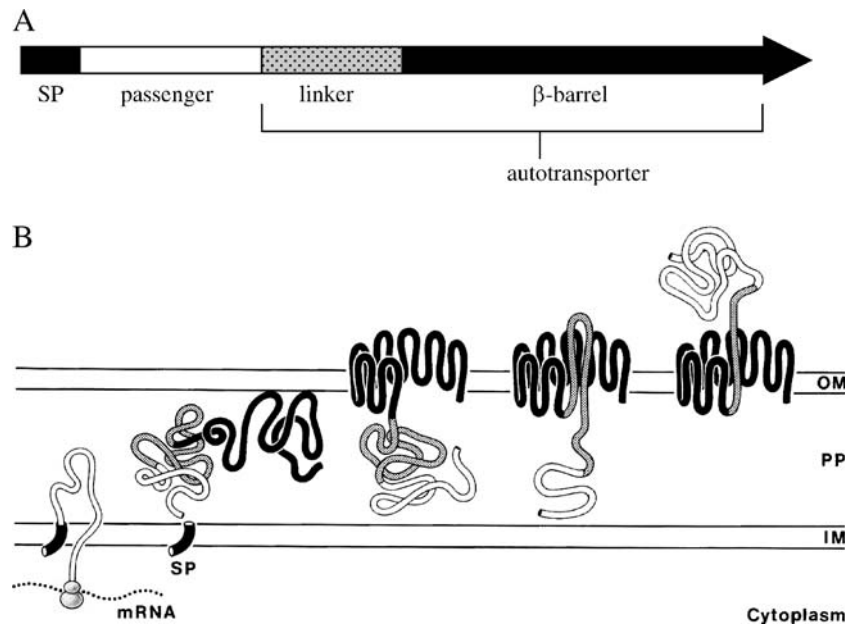
The Autodisplay system has been developed on the basis of the secretion mechanism of the autotransporter family of proteins (Jose et al. 1995). Among the different pathways, Gram-negative bacteria have evolved to transport proteins to the surface or to secrete proteins in the extracellular milieu, the autotransporter pathway is outstanding by its apparent simplicity. The autotransporters are synthesised as precursor proteins containing all structural requirements for the transport to the cell surface. IgA1 protease from *Neisseria gonorrhoeae* was the first member of this protein family that was discovered and characterized by Meyer and co-workers in the late 1980s (Pohlner et al. 1987). A concept for its secretion mechanism was proposed concurrently with its discovery (Fig. 1). It was realized soon that this secretion mechanism could be exploited for the transport of a recombinant protein in *E. coli*, by replacing the coding region for the natural passenger, the IgA1 protease, with the coding region for the recombinant protein of interest and subsequent expression and surface display in *E. coli* (Klauser et al. 1990). The proof of principle was demonstrated by several examples of recombinant polypeptides (Klauser et al. 1992; Pohlner et al. 1992), but extensive biotechnological applications failed to appear.

After the discovery of IgA1 protease from *N. gonorrhoeae* and its secretion mechanism, it was quickly concluded that some surface proteins from other Gram-negative bacteria could be transported by similar means (Klauser et al. 1993). In 1995, ten surface or secreted proteins from Gram-negative bacteria were selected by common structural features and were comprised to a new protein family called “autotransporters” (Jose et al. 1995). In this study, a protein from *E. coli*, AIDA-I, was identified to be transported likewise with IgA1 protease from *N. gonorrhoeae*. In contrast to IgA1 protease, AIDA-I occurs naturally in *E. coli* and was therefore considered a superior tool for the surface display in its homologous host. AIDA-I was initially discovered by Schmidt and co-workers (Benz and Schmidt 1989) and has been the subject of intensive studies on structure and function by this group.

For the development of the Autodisplay system, the β -barrel and the linker region of AIDA-I were combined in frame with the signal peptide of the cholera toxin β -subunit (CTB) and a strong constitutive promoter (P_{TK}) within a medium copy number plasmid backbone (Maurer et al. 1997). Into the linker regions used for Autodisplay, protease cleavage sites for the sequence specific release of the passenger protein as well as epitopes for detection by monoclonal antibodies were inserted (Maurer et al. 1999). An antibody-independent detection method, which requires only the addition of a single cysteine in the linker region, was developed for Autodisplay and was named “Cystope tagging” (Jose and Handel 2003; Jose and von Schwichow 2004b). A schematic description of the structure of a typical artificial autotransporter protein used for Autodisplay is given in Fig. 2.

Fig. 1 Secretion mechanism of the autotransporter proteins.

a Structure of the polyprotein precursor. **b** Transport of the recombinant passenger. By the use of a typical signal peptide, a precursor protein is transported across the inner membrane. Arriving at the periplasm, the C-terminal part of the precursor folds as a porin-like structure, as so-called β -barrel within the outer membrane and the passenger is transmitted to the cell surface. *SP* Signal peptide, *IM* inner membrane, *PP* periplasm, *OM* outer membrane



As mentioned above, the terminal step in Autodisplay requires the translocation of the passenger through a size-limited pore formed by the β -barrel. This means that the passenger is not allowed to acquire a stable three-dimensional conformation during transport to maintain a transport-compatible state (Klauser et al. 1990; Jose et al. 1996). In case of stable folding, transport is blocked in the periplasm (Jose and Zangen 2005). As a wide variety of passenger proteins with high biotechnological impact contain disulfide bridges and these bonds are normally formed in the periplasm of *E. coli*, a DsbA-negative mutant strain of *E. coli* (JK321) was constructed and shown to facilitate the Autodisplay of such types of proteins as well (Jose et al. 1996).

In summary, the Autodisplay system consists of vectors encoding various artificial autotransporter genes using the β -barrel from AIDA-I and different parts of its linker region. Dependent on the application, different modifications of the linker regions, various signal peptides under the control of inducible or constitutive promoters, mutant

strains of *E. coli* supporting the transport and the surface display by the autotransporter pathway and detection methods are now available that allow following surface translocation, preferentially independent of the protein domain used as a passenger.

Autodisplay of enzymes and whole cell biocatalysis

The spectrum of enzymes displayed at the cell surface by Autodisplay spans hydrolases, oxidoreductases as well as electron transfer proteins (Table 1).

Hydrolases

A first example for the surface display of an active enzyme by Autodisplay in *E. coli* was β -lactamase (Lattemann et al. 2000). In an OmpT/DsbA-negative host background,

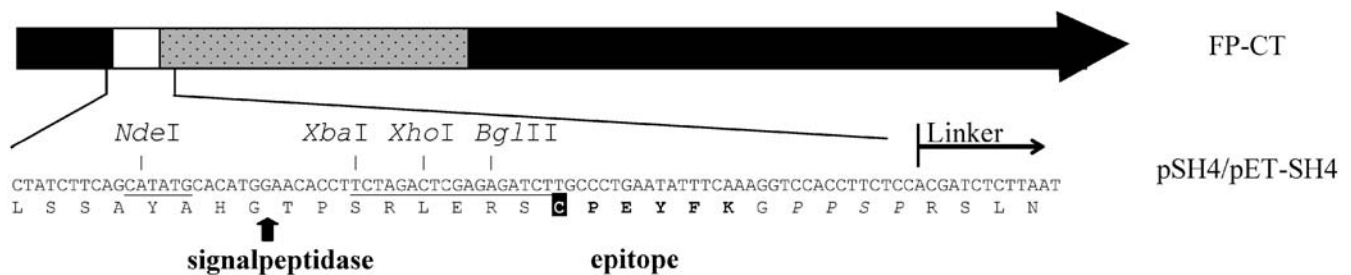


Fig. 2 Structure of a typical artificial autotransporter protein used in Autodisplay. FP-CT is a cysteine-containing fusion protein that is encoded either by plasmid pSH4 under control of a strong constitutive promoter (P_{TK}) or by plasmid pET-SH4 under the control of the inducible T7/lac promoter. The environments of the passenger insertion site, necessary to obtain surface translocation, are given as sequences. Restriction endonuclease cleavage sites for insertion of passenger-encoding DNA sequences are underlined. Various other restriction sites are available in similar plasmids. The signal peptide originates from CTB and the signal peptidase cleavage site is marked by an arrow. The

signal peptides of OmpA, β -lactamase, PelB, and AIDA-I have been used as well. The specific cleavage site for IgA1 protease that can be applied for the release of the passenger protein into the extracellular milieu is written in *italics* and the linear epitope for a mouse monoclonal antibody (*Dü142*), which can be used for labelling, is written in *bold*. The cysteine used in "Cystope tagging" a specific for labelling and detection method is indicated by a black box. *p* Plasmid, *FP* fusion protein, *SP* signal peptide, *P* passenger (Jose and Handel 2003)

Table 1 Recombinant proteins and peptides expressed at the cell surface by Autodisplay

Displayed passenger	Application	References
Cholera toxin β -subunit (CTB)	Translocation studies	(Maurer et al. 1997, 1999)
NEF epitope of HIV (PEYFK)	Translocation studies	(Maurer et al. 1997) (Jose and Handel 2003)
β -Lactamase	Translocation studies	(Lattemann et al. 2000)
Sorbitol dehydrogenase (SDH) (<i>R. sphaeroides</i>)	Whole cell biocatalysis	(Jose and von Schwichow 2004a,b)
Bovine adrenodoxin (Adx)	Steroid synthesis	(Jose et al. 2001, 2002) (Jose and Handel 2003)
	Whole cell biocatalysis	
Esterase A (<i>B. gladioli</i>)	Whole cell ester hydrolysis	(Schultheiss et al. 2002)
Aprotinin (bovine pancreatic trypsin inhibitor, BPTI)	Enzyme binding studies, translocation studies	(Jose and Zangen 2005)
P15 (human C reactive protein)	Random library screening	(Jose et al. 2005)
T cell epitopes of HSP60 (<i>Y. enterocolitica</i>)	T cell stimulation	(Konieczny et al. 2000) ^a
HSP60 _{74–86} epitope (<i>Y. enterocolitica</i>)	Vaccination	(Kramer et al. 2003)
Heat labile enterotoxin B subunit LT _B (<i>E. coli</i>)	Translocation studies	(Konieczny et al. 2000) ^a
UreA and fragments (<i>H. pylori</i>)	Vaccination	(Rizos et al. 2003)

^aNative AIDA-I was used

considerable amounts of β -lactamase were detectable at the cell surface. Cell integrity remained undisturbed, indicating a clear advantage of Autodisplay in the surface expression of β -lactamase in comparison to other display systems in *E. coli* (Francisco et al. 1992).

Esterases

Among the hydrolases, esterases represent a group of enzymes of great interest for biotechnological and industrial applications since they exhibit a broad natural variety concerning substrate and reaction type (Bornscheuer 2002). Due to their diverse substrate specificities and their stereoselectivity, esterases have been successfully used in the synthesis of optically pure substances (Reetz 2000; Schmidt et al. 2004). The first esterase that was displayed in an active form on the surface of *E. coli* was EstA from *Burgholderia gladioli* by the use of Autodisplay (Schultheiss et al. 2002). The enzyme activity of whole cells displaying EstA could be determined photometrically, by an agar plate pH-assay and by a filter overlay assay with α -naphthyl acetate as the substrate, providing access to high-throughput screening for the analysis of enzyme libraries. The viability of *E. coli* cells displaying EstA was not reduced, i.e. the number of colony forming units (CFU) remained unaltered. This is a clear advantage of the heterologous surface display of esterases by Autodisplay in comparison to other systems in which, dependent on the heterologous esterase displayed, only 15–60% of CFU were maintained (Becker et al. 2005). Autodisplay could be used in analogy to EstA for the surface display of other heterologous esterases (Jose and von Schwichow 2004b). More recently, autotransporter proteins have been discovered, which possess an esterase moiety as the natural passenger (Wilhelm et al. 1999; Lee and Byun 2003). These proteins could provide an even easier access to exploit the synthetic potential of an esterase enzyme displayed at the cell surface.

Sorbitol dehydrogenase (SDH)

Polyols and sugars are awkward to be produced by standard organic synthesis due to the high number of identical functional groups at different positions of these molecules. Hence, biocatalysis using enzymes with high regio- and stereoselectivity could afford a solution (Giffhorn et al. 2000). For this purpose SDH from *Rhodobacter sphaeroides* was expressed at the cell surface by Autodisplay (Jose and von Schwichow 2004a). SDH belongs to the short-chain dehydrogenase/reductase (SDR) family of proteins and is a dimer with a subunit molecular mass of 29 kDa (257 aa) (Philippsen et al. 2005). When sample buffer without 2-mercaptoethanol was used (non-reducing conditions) in order to analyse the surface-exposed proteins, a protein band double the size of the monomeric SDH-autotransporter fusion protein was seen in SDS-PAGE, which was recognized by the SDH-specific antiserum. This was a clear indication of a passenger-driven dimerization of monomeric SDH subunits at the cell surface (Fig. 3a). This passenger-driven dimerization is a unique feature of Autodisplay and has not been observed in any other display system until today. The whole cell biocatalyst obtained by Autodisplay of SDH could be used for the efficient synthesis of sorbitol (0.11 U/2.5 \times 10⁹ cells), fructose (0.11 U), D-tagatose (0.07 U) and L-ribulose (0.15 U) (Jose and von Schwichow 2004a).

Bovine adrenodoxin

The ferredoxin from bovine adrenal cortex, termed adrenodoxin (Adx, 14.4 kDa), belongs to the [2Fe–2S] ferredoxins, a family of small, acidic iron–sulfur proteins that can be found in bacteria, plants and animals (Grinberg et al. 2000). It plays an essential role in electron transport from adrenodoxin reductase (AdR) to mitochondrial cytochrome P450 enzymes, which are among other things involved in

display system so far (Jose and von Schwichow 2004a). **b** Whole cell biocatalyst for the synthesis of steroids obtained by Autodisplay of Adx. After transport of apo-Adx to the cell surface, dimers were formed spontaneously and the electron transfer activity of Adx was restored by chemical incorporation of the [2Fe-2S] cluster. After the addition of purified Adx reductase (*AdR*) and CYP11A1 or CYP11B1, an efficient whole cell biocatalyst for the synthesis of pregnenolone or corticosterone, respectively, was obtained. The cell envelope of *E. coli* provided sufficient membrane environment to both *P450* enzymes to be active (Jose et al. 2001, 2002)

iron-sulfur cluster (Jose et al. 2001). This result was in concordance with the concept of the autotransporter secretion mechanism, by which a protein can only be transported to the surface in an unfolded state, i.e. in case of Adx as apo-Adx without prosthetic group. We were able to activate the apo-Adx molecules displayed at the cell surface by a single-vial chemical incorporation of the [2Fe-2S] cluster under anaerobic conditions. Cells survived the procedure without loss and could be handled afterwards under aerobic conditions without reduction in activity (Jose et al. 2001).

Subsequent addition of purified AdR and *P450* CYP11A1 or *P450* CYP11B1 yielded a whole cell biocatalyst for the efficient synthesis of pregnenolone from cholesterol or corticosterone from 11-deoxycorticosterone, respectively (Fig. 3b) (Jose et al. 2002). This indicates that whole cells displaying functional Adx molecules at the surface provide sufficient environment for *P450* enzymes that are naturally membrane-associated to function. The whole cell system provides substantial improvements in accessing the biotechnological potential of *P450* enzymes. Moreover, Autodisplay of Adx is the first report on the cellular surface display of a functional recombinant protein with an incorporated inorganic prosthetic group.

Autodisplay of enzyme inhibitors and library screening

A unique feature of the Autodisplay system is its application as a tool in drug discovery (Jose et al. 2005). This challenging application is basically comprised of three steps: (a) surface display of peptide libraries, (b) labelling single cells with an inhibiting structure by target enzyme binding and (c) selecting and sorting of labelled cells by FACS. The inhibitor-target enzyme affinity can be exploited to specifically label cells of *E. coli* that display an active inhibitor at the cell surface. In case that the enzyme is coupled to a fluorescent dye, flow cytometry can be used to sort single cells labelled by this procedure and, afterwards, the selected cell can be used for clonal production of cell quantities sufficient for analytical or preparative purposes.

The first step in this strategy was to show that an enzyme inhibitor can be expressed in an active conformation at the surface of *E. coli* by Autodisplay. For this purpose, aprotinin (62 aa), a rapidly folding, three disulfide bond stabilized serine protease inhibitor (Creighton 1992), was chosen as a passenger in Autodisplay (Jose and Zangen 2005). Aprotinin has been shown earlier to be a strong inhibitor of human leukocyte elastase with a K_i of 3.5 μ M (McBride et al. 1999). It turned out that under reducing conditions, which diminished the degree of disulfide bonds formed in the periplasm, not high but detectable amounts of aprotinin appeared at the cell surface. Cells displaying aprotinin were able to specifically bind human leukocyte elastase and this binding could be quantified by flow cytometer analysis. These results showed for the first time that it is indeed possible to label cells of *E. coli* expressing an inhibitor at the cell surface by the affinity of this inhibitor to the target enzyme (Jose and Zangen 2005).

In a following study, P15, a protease inhibitor without disulfide bond stabilization was investigated as a model inhibitor in Autodisplay (Jose et al. *in press*). P15 consists of fifteen amino acids and is derived from the human C-reactive protein, whose serum concentration increases during acute inflammation. It has been shown to be a strong inhibitor of human cathepsin G with a K_i of 0.25 μ M (Yavin et al. 1996). Due to its less stable three-dimensional structure, P15 could be expressed in higher amounts than aprotinin at the cell surface of *E. coli* by Autodisplay and

cells displaying P15 could be efficiently labelled with human cathepsin G which was coupled to FITC (Jose et al. 2005). This second example for a successful target enzyme labelling of cells displaying an inhibitor could be easily quantified by FACS. Moreover, cells displaying P15 could be sorted from a mixed population with an excess of control cells displaying a non-inhibiting peptide. The selected cells survived this procedure without loss and were grown to single-cell clones that could be subjected to DNA sequence analysis and studies on protein expression. This method was subsequently used to screen a random surface display library consisting of 1.15×10^5 different variants and three new peptide inhibitors of cathepsin G were identified (Jose et al. 2005). It turned out that Autodisplay of an enzyme inhibitor with subsequent target enzyme labelling and FACS is a reliable method to sort cells displaying an active inhibitory structure at the surface. By repeated cycles of surface display of random libraries and sorting cells by an increased fluorescence due to target enzyme binding, a gradual improvement and optimisation is possible, which is congruent with the idea of directed evolution.

Autodisplay of epitopes and vaccine development

Besides the surface display of enzymes and enzyme inhibitors, the cellular surface display of epitopes and antigens is a promising application of Autodisplay. In addition, it has been shown in earlier studies on the development of whole cell vaccines that the secretion of antigens results in an improved immune answer compared to intracellular-expressed antigens (Hess et al. 1996). Therefore, the advantages of Autodisplay come along with enhanced stimulation of the immune system in order to generate live oral vaccine vectors as vaccine carriers. Regarding this perspective, the NEF epitope of the human immunodeficiency virus was one of the two initial passenger proteins beside CTB that have been used to establish the Autodisplay system (Maurer et al. 1997). Also, T cell epitopes of the *Yersinia enterocolitica* heat-shock protein 60 (HSP60) have been expressed at the surface of attenuated strains of *Salmonella* vaccine strains (Kramer et al. 2003) and *E. coli* by Autodisplay (Konieczny et al. 2000). T cells isolated from mice immunized with *Salmonella* cells displaying HSP60 exhibited an antigen-specific proliferation accompanied by high-level IFN- γ secretion. This indicates an effective immune stimulation by the vaccine strains orally applied to C57BL/6 mice and demonstrates the applicability of Autodisplay of antigenic determinants for live-attenuated *Salmonella* vaccine strains development. More recently, Autodisplay was used for the expression of antigenic urease fragments from *Helicobacter pylori* on the surface of *Salmonella entericaserovar typhimurium* vaccine strains and the subsequent oral immunization of specific-pathogen-free female BALB/c mice (Rizos et al. 2003). This resulted in a significant reduction in the *H. pylori* burden after challenge infection and, hence, to an increased protective efficacy in the murine *Helicobacter*

infection model. Taken together, these results indicate that Autodisplay increases the immunogenicity of recombinant antigens expressed from oral live vaccine strains and show the feasibility of immunizing against *H. pylori* with *Salmonella* vaccine strains displaying T cell epitopes by Autodisplay.

Summary and future applications of the Autodisplay system

Autodisplay is an easy-to-handle system for the surface display of recombinant proteins. It has some striking advantages in comparison to other surface display systems applied so far.

The first advantage is the high number of recombinant proteins displayed at the surface of *E. coli*. The number of recombinant molecules was determined to be in the range of $1.5\text{--}1.8 \times 10^5$ per single cell based on the electron transfer activity of bovine Adx (Jose et al. 2001) or a specific labelling procedure of SDH from *Rhodobacter sphaeroides* (Jose and von Schwichow 2004a). A crucial point in order to display a recombinant protein at the cell surface in adequate numbers with high reproducibility is that passenger proteins need to remain in an unfolded conformation to maintain a transport-competent state (Klauser et al. 1990; Jose et al. 1996; Jose and Zangen 2005).

A second striking advantage of the Autodisplay system is the free motility of the β -barrel serving as an anchor for the recombinant protein within the outer membrane. This enables passenger proteins, which are expressed as monomers, to form functional dimers at the cell surface, as observed in the Autodisplay of Adx and SDH (Jose et al. 2001; Jose and von Schwichow 2004a). The system could also be used for the surface expression of heterodimeric proteins such that each monomer is expressed as a single fusion with the domains needed for Autodisplay. It cannot be excluded that higher degrees of association can be obtained at the cell surface by Autodisplay, e.g. trimers, tetramers or even hexamers. This is under current investigation.

Another outstanding characteristic of the Autodisplay system is the opportunity to incorporate an inorganic prosthetic group in an apo-protein expressed at the cell surface of *E. coli*, as it was successfully applied to the [2Fe-2S] cluster of bovine Adx (Jose et al. 2001, 2002). The functional surface display of proteins that require inorganic cofactors has not been reported by any other surface display system than Autodisplay so far.

Finally, the most convenient feature of Autodisplay is that the surface expression of recombinant proteins up to numbers beyond 10^5 molecules per single cell of *E. coli* is not reducing cell viability or cell integrity (Maurer et al. 1997; Jose et al. 2001, 2005; Jose and von Schwichow 2004a). This enabled, e.g., the cells to survive without restrictions challenging analytic approaches with whole cells including FACS. Especially, the latter enables to pick single cells with a distinct feature from a library of variants, fol-

lowed by the clonal expansion for sequence determination or structure analysis of the molecule displayed at the surface.

All in all, the Autodisplay system has proven to be a very efficient tool for the surface display of a wide variety of recombinant proteins in *E. coli* or perhaps, generally, in Gram-negative bacteria. Until today, its applications span the functional surface display of enzymes, enzyme inhibitors, antigens or receptors, the development of whole cell biocatalysts or biofactories, as well as the peptide library screening by target enzyme binding. Future experiments will try out its further application in analytical or preparative purposes.

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References

- Becker S, Theile S, Heppeler N, Michalczyk A, Wentzel A, Wilhelm S, Jaeger KE, Kolmar H (2005) A generic system for the *Escherichia coli* cell-surface display of lipolytic enzymes. *FEBS Lett* 579:1177–1182
- Benhar I (2001) Biotechnological applications of phage and cell display. *Biotechnol Adv* 19:1–33
- Benz I, Schmidt MA (1989) Cloning and expression of an adhesin (AIDA-I) involved in diffuse adherence of enteropathogenic *Escherichia coli*. *Infect Immun* 57:1506–1511
- Bessette PH, Rice JJ, Daugherty PS (2004) Rapid isolation of high-affinity protein binding peptides using bacterial display. *Protein Eng Des Sel* 17:731–739
- Bornscheuer UT (2002) Microbial carboxyl esterases: classification, properties and application in biocatalysis. *FEMS Microbiol Rev* 26:73–81
- Charbit A, Boulain JC, Ryter A, Hofnung M (1986) Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope: expression at the cell surface. *EMBO J* 5:3029–3037
- Creighton TE (1992) The disulfide folding pathway of BPTI. *Science* 256:111–114
- Francisco JA, Earhart CF, Georgiou G (1992) Transport and anchoring of beta-lactamase to the external surface of *Escherichia coli*. *Proc Natl Acad Sci USA* 89:2713–2717
- Francisco JA, Campbell R, Iverson BL, Georgiou G (1993) Production and fluorescence-activated cell sorting of *Escherichia coli* expressing a functional antibody fragment on the external surface. *Proc Natl Acad Sci USA* 90:10444–10448
- Freudl R, MacIntyre S, Degen M, Henning U (1986) Cell surface exposure of the outer membrane protein OmpA of *Escherichia coli* K-12. *J Mol Biol* 188:491–494
- Georgiou G, Stathopoulos C, Daugherty PS, Nayak AR, Iverson BL, Curtiss R, 3rd (1997) Display of heterologous proteins on the surface of microorganisms: from the screening of combinatorial libraries to live recombinant vaccines. *Nat Biotechnol* 15:29–34
- Giffhorn F, Kopfer S, Huwig A, Freimund S (2000) Rare sugars and sugar-based synthons by chemo-enzymatic synthesis. *Enzyme Microb Technol* 27:734–742
- Grinberg AV, Hannemann F, Schiffler B, Muller J, Heinemann U, Bernhardt R (2000) Adrenodoxin: structure, stability, and electron transfer properties. *Proteins* 40:590–612
- Hess J, Dreher A, Gentschev I, Goebel W, Ladel C, Miko D, Kaufmann SH (1996) Protein p60 participates in intestinal host invasion by *Listeria monocytogenes*. *Zentralbl Bakteriol* 284:263–272
- Jose J, Handel S (2003) Monitoring the cellular surface display of recombinant proteins by cysteine labeling and flow cytometry. *Chembiochem* 4:396–405

- Jose J, von Schwichow S (2004a) Autodisplay of active sorbitol dehydrogenase (SDH) yields a whole cell biocatalyst for the synthesis of rare sugars. *Chembiochem* 5:100–108
- Jose J, von Schwichow S (2004b) "Cysteine tagging" for labeling and detection of recombinant protein expression. *Anal Biochem* 331:267–274
- Jose J, Zangen D (2005) Autodisplay of the protease inhibitor aprotinin in *Escherichia coli*. *Biochem Biophys Res Commun* 333:1218–1226
- Jose J, Jahnig F, Meyer TF (1995) Common structural features of IgA1 protease-like outer membrane protein autotransporters. *Mol Microbiol* 18:378–380
- Jose J, Kramer J, Klauser T, Pohlner J, Meyer TF (1996) Absence of periplasmic DsbA oxidoreductase facilitates export of cysteine-containing passenger proteins to the *Escherichia coli* cell surface via the Iga beta autotransporter pathway. *Gene* 178:107–110
- Jose J, Hannemann F, Bernhardt R (2001) Functional display of active bovine adrenodoxin on the surface of *E. coli* by chemical incorporation of the [2Fe–2S] cluster. *Chembiochem* 2:695–701
- Jose J, Bernhardt R, Hannemann F (2002) Cellular surface display of dimeric Adx and whole cell *P450*-mediated steroid synthesis on *E. coli*. *J Biotechnol* 95:257–268
- Jose J, Betscheider D, Zangen D (2005) Bacterial surface display library screening by target enzyme labelling: identification of new human cathepsin G inhibitors. *Anal Biochem* 346:258–267
- Klauser T, Pohlner J, Meyer TF (1990) Extracellular transport of cholera toxin B subunit using *Neisseria* IgA protease beta-domain: conformation-dependent outer membrane translocation. *EMBO J* 9:1991–1999
- Klauser T, Pohlner J, Meyer TF (1992) Selective extracellular release of cholera toxin B subunit by *Escherichia coli*: dissection of *Neisseria* IgA beta-mediated outer membrane transport. *EMBO J* 11:2327–2335
- Klauser T, Pohlner J, Meyer TF (1993) The secretion pathway of IgA protease-type proteins in gram-negative bacteria. *Bioessays* 15:799–805
- Konieczny MP, Suhr M, Noll A, Autenrieth IB, Alexander Schmidt M (2000) Cell surface presentation of recombinant (poly-) peptides including functional T-cell epitopes by the AIDA autotransporter system. *FEMS Immunol Med Microbiol* 27:321–332
- Kramer U, Rizos K, Apfel H, Autenrieth IB, Lattemann CT (2003) Autodisplay: development of an efficacious system for surface display of antigenic determinants in *Salmonella* vaccine strains. *Infect Immun* 71:1944–1952
- Lattemann CT, Maurer J, Gerland E, Meyer TF (2000) Autodisplay: functional display of active beta-lactamase on the surface of *Escherichia coli* by the AIDA-I autotransporter. *J Bacteriol* 182:3726–3733
- Lee HW, Byun SM (2003) The pore size of the autotransporter domain is critical for the active translocation of the passenger domain. *Biochem Biophys Res Commun* 307:820–825
- Lee SY, Choi JH, Xu Z (2003) Microbial cell-surface display. *Trends Biotechnol* 21:45–52
- Maurer J, Jose J, Meyer TF (1997) Autodisplay: one-component system for efficient surface display and release of soluble recombinant proteins from *Escherichia coli*. *J Bacteriol* 179:794–804
- Maurer J, Jose J, Meyer TF (1999) Characterization of the essential transport function of the AIDA-I autotransporter and evidence supporting structural predictions. *J Bacteriol* 181:7014–7020
- McBride JD, Freeman HN, Leatherbarrow RJ (1999) Selection of human elastase inhibitors from a conformationally constrained combinatorial peptide library. *Eur J Biochem* 266:403–412
- Philippsen A, Schirmer T, Stein MA, Giffhorn F, Stetefeld J (2005) Structure of zinc-independent sorbitol dehydrogenase from *Rhodobacter sphaeroides* at 2.4 Å resolution. *Acta Crystallogr D Biol Crystallogr* 61:374–379
- Pikuleva IA, Tesh K, Waterman MR, Kim Y (2000) The tertiary structure of full-length bovine adrenodoxin suggests functional dimers. *Arch Biochem Biophys* 373:44–55
- Pohlner J, Halter R, Beyreuther K, Meyer TF (1987) Gene structure and extracellular secretion of *Neisseria gonorrhoeae* IgA protease. *Nature* 325:458–462
- Pohlner J, Klauser T, Kuttler E, Halter R (1992) Sequence-specific cleavage of protein fusions using a recombinant *Neisseria* type 2 IgA protease. *Biotechnology (NY)* 10:799–804
- Reetz MT (2000) Evolution in the test tube as a means to create enantioselective enzymes for use in organic synthesis. *Sci Prog* 83:157–172
- Rizos K, Lattemann CT, Bumann D, Meyer TF, Aebischer T (2003) Autodisplay: efficacious surface exposure of antigenic UreA fragments from *Helicobacter pylori* in *Salmonella* vaccine strains. *Infect Immun* 71:6320–6328
- Schmidt M, Baumann M, Henke E, Konarzycka-Bessler M, Bornscheuer UT (2004) Directed evolution of lipases and esterases. *Methods Enzymol* 388:199–207
- Schultheiss E, Paar C, Schwab H, Jose J (2002) Functional esterase surface display by the autotransporter pathway in *Escherichia coli*. *J Mol Catal B Enzym* 18:89–97
- Smith GP (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228:1315–1317
- Strauss A, Gotz F (1996) In vivo immobilization of enzymatically active polypeptides on the cell surface of *Staphylococcus carnosus*. *Mol Microbiol* 21:491–500
- Wentzel A, Christmann A, Kratzner R, Kolmar H (1999) Sequence requirements of the GPNG beta-turn of the *Ecballium elaterium* trypsin inhibitor II explored by combinatorial library screening. *J Biol Chem* 274:21037–21043
- Wernerus H, Stahl S (2004) Biotechnological applications for surface-engineered bacteria. *Biotechnol Appl Biochem* 40:209–228
- Wilhelm S, Tommassen J, Jaeger KE (1999) A novel lipolytic enzyme located in the outer membrane of *Pseudomonas aeruginosa*. *J Bacteriol* 181:6977–6986
- Yavin EJ, Yan L, Desiderio DM, Fridkin M (1996) Synthetic peptides derived from the sequence of human C-reactive protein inhibit the enzymatic activities of human leukocyte elastase and human leukocyte cathepsin G. *Int J Pept Protein Res* 48:465–476